Table 1. Estrogenic activity of some isoflavone derivatives.

No. of mice	Treatment	Avg. uterine weight (mg)	Approx. potency*
6	Normal control	6.4 ± 0.8†	
6	2.5 mg biochanin A	20.9 ± 3.1	0.033
5	2.5 mg daidzein	26.6 ± 4.1	.042
6	2.5 mg formononetin	8.9 ± 1.2	.009
5	2.5 mg genistein	19.3 ± 1.3	.030
6	0.01 μg stilbestrol	9.4 ± 0.8	
6	0.02 μg stilbestrol	15.7 ± 1.5	
6	0.04 μg stilbestrol	22.2 ± 2.1	
5	0.08 μg stilbestrol	46.2 ± 4.7	

* Expressed as micrograms of diethylstilbestrol activity.
† Standard deviation.

the least estrogenic activity. From the structural formula, it can be seen that formononetin is the only compound tested that has only one free hydroxyl group. Since the activity in many synthetic estrogenic compounds is known to be related to the number and arrangement of hydroxyl groups (*8*), it is not surprising that formononetin is less active than the other compounds tested.

Biochanin A was recently isolated from red clover and found to be estrogenic (*9*). The isoflavone daidzein has not been reported as being present in nature. However, its glucoside, daidzein, has been isolated from soybean-oil meal (*10*).

It appears likely that the estrogenic activity in livestock feeds is primarily due to compounds classified chemically as isoflavone derivatives. The estrogenic potency of these isoflavone compounds is low when compared with that of diethylstilbestrol; nevertheless, when one considers the large amount of such feeds as legume hay and soybean-oil meal that are consumed by farm animals, enough estrogenic substance may be present in these feeds to exert an important influence upon their physiological functions. The effect may be adverse if too much of the estrogenic substance is present, as in the case of subterranean clover (*2*). On the other hand, if the correct amount is present, the effect may be as beneficial as diethylstilbestrol in stimulating live-weight gain in beef cattle as shown by Burroughs *et al.* (*1*).

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Antiaccelerator and Antiarrhythmic Cardiac Action of Synthetic Steroid Alkamines

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The demonstration of antiaccelerator cardiac activity for the synthetic steroid secondary amine, 20-(5′methyl-2′-piperidyl)-5-pregnen-3,20-diol (*1, 2*), prompted the synthesis of several steroids with alkamine substitutions in the 16 position by D. Gould and E. B. Hershberg in the Chemical Research Division of Schering Corporation. Our objective was a potent antiaccelerator agent suitable for studies in human beings.

Antiaccelerator cardiac action was first observed in our series with Sch 1837, 16-N-piperidinopregnenolone. This substance and closely related derivatives are strongly convulsant in nonanesthetized dogs at antiaccelerator dosages. However, the activity in this structure encouraged further synthetic efforts, and pharmacological studies brought attention to cyclohexylamine derivatives of pregnenolone. Sch 2331, 16-cyclohexylaminopregnenolone, displayed antiaccelerator activity, and antagonized experimentally induced auricular and ventricular arrhythmias. Sch 2602, 16-cyclohexylaminopregnandiol, was highly effective as an antiaccelerator and antiarrhythmic agent, but it disclosed no important advance over Sch 2331 from a toxicity standpoint. Although these two compounds provided a distinct advance over earlier preparations and could display cardiac activity in nonanesthetized dogs, they induced central stimulation at doses only slightly above those causing antiaccelerator or antiarrhythmic activity. Further experiments demonstrated the greater activity of Sch 2684, 16-cyclohexylamino-allopregnandiol, and indicated its relative safety. Sch 2331, Sch 2602, and Sch 2684 present a new active chemical series with a noteworthy pharmacological combination of highly effective cardiac antiaccelerator and antiarrhythmic action.

The antiaccelerator effect of Sch 1837 and Sch 2331 was suggested by the observation of a distinct slowing of heart rate after injection of these substances into normal and atropinized anesthetized dogs. The compound prepared by Uhle (*1*) and Sch 2684 behave similarly in such tests. Evidence for a direct cardiac action was obtained with isolated perfused rabbit hearts (Langendorff preparation) and by dog heart-lung experiments (after Krayer, *3*). The positive chronotropic action of epinephrine on isolated rabbit hearts was partially or completely blocked by 5 to 50 μg per heart of Sch 2684 without diminishing the positive inotropic response to the epinephrine. In the dog heart-lung preparations, 7 mg of Sch 2684 in a single dose blocked the cardiac acceleration induced by a continuous infusion of epinephrine hydrochloride at a rate of 10 to 45 μg/min, whereas the cardiac output remained essentially unaltered. Marked slowing of the pulse rate in preliminary clinical trials with Sch 2684

appears to parallel the antiaccelerator behavior of the compound in dog studies.

Antiarrhythmic activity was observed in experimentally induced auricular and ventricular arrhythmias. Auricular fibrillation induced by local application of Mecholyl chloride to the sinus-node region accompanied by mechanical pinching (*4*) was consistently arrested or prevented by intravenous injection of Sch 2684 (1.5 to 4.0 mg/kg) in anesthetized, vagotomized open-chest dogs. Intravenous quinidine sulfate arrested or prevented the arrhythmias at 2 to 4 mg/kg. Protection against the induction of arrhythmias was not as complete as with Sch 2684, and higher doses (10 mg/kg) of quinidine precipitated ventricular tachycardia and fibrillation in some animals. No ventricular tachycardia or fibrillation was observed after Sch 2684; in rare instances, transient premature ventricular contractions were found.

Auricular arrhythmias induced in dog preparations by subepicardial injection of aconitine nitrate at the sinus-node area (*5*) were antagonized by 2 to 4 mg/kg of Sch 2684 given intravenously; moderate or marked slowing of the ventricular rate, sometimes accompanied by a partial or full reappearance of P waves, occurred and indicated a diminution in the rate of formation of abnormal impulses. Under comparable conditions, intravenous quinidine sulfate (4 to 8 mg/kg) in divided doses produced only partial reappearance of P waves and inconsistent slowing of the heart rate.

In experimental ventricular tachycardia induced by intravenous ouabain (*6*) in nonanesthetized dogs, consistent slowing of the ventricular rate with complete reversion to sinus rhythm occurred in approximately one-half of the tests following intravenous Sch 2684 (2 to 4 mg/kg) in single or divided doses. In comparable tests, intravenous procaine amide hydrochloride at 4 to 12 mg/kg in divided doses caused partial reappearance of P waves and slowing of the ventricular rate; complete abolition of the arrhythmia occurred at 12 to 28 mg/kg. Ventricular tachycardia induced in nonanesthetized dogs by ligation of the anterior descending branch of the left coronary artery (*7*) was slowed by 4 to 6 mg/kg of Sch 2684 intravenously, and sinus rhythm reappeared following 6 to 10 mg/kg. Under similar conditions, procaine amide generally restores sinus rhythm at 20 mg/kg given intravenously.

In electrocardiographic studies, Sch 2684 failed to cause any significant changes in the ECG (Standard Limb Lead II) pattern after the injection of antiaccelerator or antiarrhythmic dosages. Following repeated 4-mg/kg injections of Sch 2684 to anesthetized dogs during approximately 10 min, these changes were present: (i) prolongation of the measured P-R interval in most tests (after 8 to 12 mg/kg), but P-R remained essentially unaltered as correlated with changes in heart rate; (ii) lengthening of both the measured and the relative Q-T duration as expressed by the Q-T ratio following 8 to 12 mg/kg; (iii) increase in the duration of the Q-R-S complex after 4 to 12 mg/kg; (iv) further decrease in the amplitude of the R wave and increased amplitude of the S wave; (v) gradual increase of T wave voltage initially and then slight decline; (vi) decrease in heart rate with or without initial slight transient (1 to 2 min) increase. The changes generally magnified with increasing amounts of the compound.

The selectivity of Sch 2684 is indicated by the absence of: significant antihistaminic activity, antispasmodic action on the gastrointestinal tract, classical anticholinergic action, central stimulating or emetic effects at antiaccelerator or antiarrhythmic dose levels. The compound does not block the pressor response to epinephrine or cause epinephrine reversal in blood-pressure experiments. The contraction of the cat nictitating membrane produced by faradic stimulation of preganglionic fibers of the superior cervical ganglion is not influenced by the intravenous injection of 8 mg/kg of Sch 2684. The substance appears to possess a rather selective adrenolytic (antiaccelerator) effect against epinephrine in the heart. The site of antiaccelerator action might reside at the sinus node. The mechanism of the antiarrhythmic action remains unclear, although it may be related to the adrenolytic effect on the heart.

The clinical study of Sch 2684 in cardiac arrhythmias that have responded to quinidine sulfate or procaine amide hydrochloride (*8*) appears to merit consideration. The presence of cardiac antiaccelerator properties suggests specific application in sinus tachycardia and cautious trial in the management of cardiac acceleration occurring with myocardial infarction or essential hypertension.

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